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CORRELATIONS BETWEEN VITELLINE MEMBRANE STRENGTH AND SELECTED PHYSICAL PARAMETERS OF POULTRY EGGS

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Abstract

The aim of this study was to evaluate the strength of vitelline membrane and its correlation with other morphological traits and the viscosity of egg yolk of different poultry species: goose, turkey, Muscovy duck, chicken, guinea fowl and Japanese quail. Vitelline membrane strength (VMS) was defined as work and force after the deformation of yolk at 6 mm. Bird species affected the VMS of egg yolk. The lowest strength was measured for the vitelline membrane of goose egg yolk. There were no apparent differences in the strength of vitelline membrane for ducks, guinea fowl, chickens and Japanese quail. In contrast, the vitelline membrane of turkey egg yolk appeared to be of the highest strength. Statistically significant positive correlations were observed between VMS and yolk index, while there was no correlation between the weight of the eggs and egg yolk. The work deformation of yolk was positively correlated with the viscosity of poultry egg yolk.

Key words: egg yolk, vitelline membrane strength, yolk index, viscosity

A vitelline membrane is an integral part of egg yolk, separating it from egg white (albumen). By surrounding slurry yolk, it gives a spherical shape. The structure of vitelline membrane integrity is of high biological importance. It protects the inner part of yolk against pathogens and allows the proper course of embryogenesis. During the incubation embryonic cells are responsible for structural alterations in the vitelline membrane which weaken the membrane and subsequently cause it to rupture over the embryo. After 48 h of incubation, the mechanical strength of the membrane is decreased by 73%. The factors responsible for weakening of the membrane during development have not been determined. Romanoff and Romanoff (1949) suggested that diffusion of water from the albumen into the yolk during incubation of eggs causes the yolk to swell, stretching and weakening the vitelline membrane. Loss of strength may be due to the loss of a layer from the membrane or digestion of the membrane by a tryptic protease located in the albumen.

Physical-mechanical properties of egg quality greatly influence the efficiency of food processing, such as the separation of albumen and yolk by mechanical shelling. An important parameter for an egg processing plant is the vitelline membrane strength (VMS). A strong membrane is useful for easy separation of albumen and yolk, whereas a weak membrane leads to significant economic losses because once the membrane is broken, yolk pollutes the albumen. Even a small amount of yolk reduces the foaming properties of albumen, leading to huge financial losses. Thus, it is very important to avoid albumen contamination with yolk (Berardinelli et al., 2008; Galea, 2011).

The research has shown that the VMS of yolk evaluated on the basis of its breaking strength depends on storage time and conditions (Keener et al., 2006). According to Kirunda and McKee (2000), it is associated with the process of water penetration into the yolk and with changes in the viscosity of albumen and yolk mass. The VMS has also been shown to depend on vitamin E supplementation (Kirunda et al., 2001), on chicken origin (Berardinelli et al., 2008) as well as on cage or cage-free rearing system (Jones et al., 2010). There has been no significant impact of washing egg and washing temperature on the strength of vitelline membrane (Caudill et al., 2010). The literature lacks data on the VMS of yolk of poultry species other than chicken. This is probably due to the production niche of these eggs on the world market. However, the production of eggs of other poultry species amounts to 86 billion pieces per year (FAO, 2011) and there has been a growing trend in this regard. It appears that this may be related to the increasing incidence of allergic reactions to eggs in infants and intolerance in adults (Cantani, 2008). Moreover, studies comparing the quality characteristics of eggs from different species of poultry have not demonstrated that chicken eggs outperform the other ones in terms of nutritional value and shelf life (Kaźmierska et al., 2005; Song et al., 2010; Polat et al., 2013). Thus, it seems reasonable to investigate whether differences exist in the VMS of egg yolk between different species of poultry.

The difficulty in comparing VMS values of eggs from different species of poultry (and demonstrating any correlation with other selected egg characteristics) results from a large number of variables, which also limits the ability to use conventional statistical methods. Furthermore, the excessive number of variables complicates sound inference. Thus, the results were developed using the Principal Component Analysis (PCA). This method makes it possible to study the relationships between hierarchical variables and their graphical presentation in the form of data points (retaining a maximum amount of information), whose mutual arrangement is the result of the analysis.

The aim of this study was to evaluate the strength of vitelline membrane and its correlation with other morphological traits and the apparent viscosity of egg yolk of different poultry species: goose, turkey, Muscovy duck, chicken, guinea fowl and Japanese quail.

Material and methods

The study was conducted on fresh eggs of the following bird species: White Kołuda goose W11 (G), Bronze turkey (T), Muscovy duck (MD), ISA Brown hen (H), Essor Slim guinea fowl (GF), Japanese quail (JQ) (Table 1). Fifteen eggs from each species were obtained from poultry farms located in Poland, which are under constant veterinary supervision.

All the flocks of birds were kept and fed intensively in accordance with the standards developed for the technology group. At the time of egg collection the flocks were at their top egg laying, and their production results did not differ from the established norms.

Eggs were selected randomly from the daily harvest. The eggs that significantly differed in size, with shell flaws, an unusual shape and with visible dirt were not taken into consideration. After the harvest, the eggs were delivered to the laboratory. During transport eggs were packaged in foam cartons, then placed in plastic open-sided baskets. Transport was with an average inside temperature of 12±3°C. The truck equipped with shock absorption (Air Leveling Module), drove with an average speed of 50 km/h, on a paved road in Poland. Turkey, hen, guinea fowl and Japanese quail eggs were transported no more than 15 km distance, Muscovy ducks and goose eggs 280 and 270 km, respectively. After delivery to laboratory eggs were placed in a vertical position (with air cell faced upwards) under cooling conditions at 4°C for 48 h.

The eggs were numbered and then weighed (WE) on an electronic balance to determine their masses (±0.01 g). Each egg was broken and the yolk was separated from the albumen (WY) and weighed (±0.01 g) and their height and width were also measured (± 0.01 mm).

The yolk index (Ix) was determined by measuring the width of the yolk with dial calipers and the height of the yolk using a standard electronic caliper with an accuracy of ± 0.01 mm. Yolk index = yolk height/yolk width (Funk, 1948).

The apparent viscosity of yolk (VY) in quadruplicate was determined using a Brookfield viscometer (Model DV-III, Stoughton, MA, U.S.A.). The measurements were carried out with an increasing shear rate (from 1.7 to 60 s^{-1}) and the temperature of 5°C.

Vitelline Membrane Strength (VMS) was measured using the TA.HDPlus Texture Analyzer with a 5-kg load cell, and a sensitivity of 0.1 g (or texture analyzer trigger force). Deformation speed was 3.2 $mm \cdot s^{-1}$ (Berardinelli et al., 2008). In particular, the vitelline membrane-yolk system strength was evaluated by driving a ½ call ball, stainless steel probe into the highest point of the yolk. Vitelline membrane-yolk system strength (VMS) was defined as work (mJ) and force (g) after yolk deformation at 6 mm.

The study and statistical inference were performed at the critical significance level of $P<0.05$. For all designated variables/parameters the compatibility of their distribution with a normal distribution using the Kolmogorov-Smirnov test with Lillefors correction has been verified. The homogeneity of variance has been checked by Barletta, Cochran and Levene tests (Stanisz, 2007). The following descriptive statistics were determined: the arithmetic mean, the confidence interval for the mean, median, minimum, maximum, range, standard deviation and variation (classic) coefficient. In addition, both upper and lower quartiles, quartile deviation, and the variation coefficient of position were computed for the selected parameters. The analysis of the results obtained for each egg yolk of selected poultry species was based (due to the presence of outliers) on position measures (particularly in the second quartile) and illustrated by a box-and-whisker plot. Whiskers illustrate the maximum and minimum values, the box – both top and bottom quartiles, and the square inside the box – the median. Duncan's test was used to verify the differences between the average values of the parameters of a normal distribution. Significance of differences between mean values of the parameters of the distribution different from the normal or of heterogeneous variance has been checked by Kruskal-Wallis nonparametric test. Principal Component Analysis (PCA) was performed as well. PCA is based on the use of fundamental concepts in statistics, like, among others, correlation and variance. This is the straight-line method which allows finding new axis (components) that better "describe" data, e.g. in terms of their logical groupings. The method of PCA involves making transformation of initial parameters in the set of new, mutually independent parameters (principal components, PC) (Stanisz, 2007). In order to clarify the structure of the links between the observable parameters and to reduce their number to a smaller, in this case from 5 to 2, and to find a set of common factors (principal components) and to define their relationship with observable parameters the PCA was used. Significance of differences between mean values of the parameters of the distribution different from the normal or of heterogeneous variance has been checked by Kruskal-Wallis nonparametric test (Gramacki and Gramacki, 2009). The statistical analysis was conducted using the Software System Statistica, version 10 (Statsoft Inc. 2011).

Table 1. Characteristics of strain, laying time and rearing system of poultry species

Poultry species	Strain	Laying season	Age (wk)	Wk of lay[1]	Rearing system
Goose (G)	White Kołuda; W11	Second	61	6	On litter
Turkey (T)	Bronze	First	34	4	On litter
Muscovy duck (MD)	Canedins R7IM	Second	71	7	On litter
Hen (H)	ISA Brown	First	26	7	Cages
Guinea fowl (GF)	Essor Slim	First	39	8	Cages
Japanese quail (JQ)	WT – laying type	First	11	5	Cages

[1]Counted from the start of lay in a particular laying season.

Results

As expected, significant differences (Kruskal-Wallis test, $P<0.5$) were observed in the weight of eggs (WE) and of yolk (WY) between the analyzed poultry species (Table 2). Goose egg yolk (G) was the heaviest and the lightest was that of Japanese quail (JQ). Muscovy duck eggs (MD) were characterized by a significantly high percentage of yolk weight in the weight of the entire egg in comparison to the eggs of other poultry species. The smallest share of the yolk was found in hen eggs (H) (Table 2).

Table 2. The mean values of the measured parameters of egg yolk in different species of poultry including standard deviations

Poultry species	Egg weight (g)	Yolk weight (g)	Yolk content (%)	Apparent viscosity (Pa·s)
Goose (G)	141.02±9.70 a	42.58±6.67 a	30.06±2.95 ab	2.74±0.11 a
Turkey (T)	82.64±2.57 b	26.77±4.46 b	32.27±4.40 a	2.40±0.29 a
Muscovy duck (MD)	73.67±2.32 b	27.4±2.43 b	37.15±2.34 d	1.74±0.08 b
Hen (H)	57.04±2.08 c	14.25±0.60 c	25.00±0.35 c	1.10±0.00 c
Guinea fowl (GF)	47.03±1.59 c	13.56±0.62 c	28.85±0.72 b	2.00±0.16 b
Japanese quail (JQ)	12.02±0.41 d	3.93±0.21 d	32.74±0.78 a	0.80±0.01 c

Explanatory notes: mean values designated by the same letters do not differ statistically significantly ($P<0.05$, $n = 15$).

Table 2 lists values of the average apparent viscosity of poultry egg yolk determined from 4 measurements at a shear rate of 22.8 $1 \cdot s^{-1}$. Egg yolk of different poultry species was characterized by high viscosity, which followed a decreasing order: goose, turkey> guinea fowl, duck> hen, quail. The viscosity of yolks decreased with an increasing shear rate (Figure 1), which indicates that yolks exhibited shear-thinning behavior. Such yolk properties result from yolk texture that is affected by weak physical bonds and hydrophobic interactions.

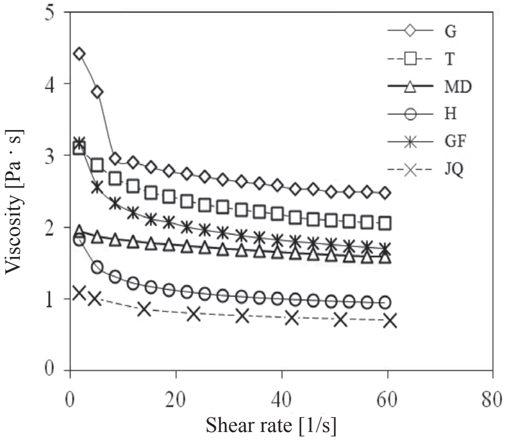

Figure 1. Viscosity of yolk of poultry eggs

Yolk index value (Ix) for eggs was depicted by the box graph in order to illustrate the variability of this parameter (Figure 2). Japanese quail egg yolk index (JQ) was the most diverse. Ix median for quail eggs was 0.48, and 75% of yolk had Ix at 0.60, while 25% at 0.46, respectively. Goose egg yolk index (G) was the lowest, and its middle value (median) was equal to 0.35, and 75% of egg yolk had Ix below 0.39 and 25% above 0.33. Comparison of the mean Ix values using the Kruskal-Wallis test ($P<0.05$) showed that only the Ix of goose eggs (G) and Muscovy duck eggs (MD) was significantly different from the Ix of other poultry species.

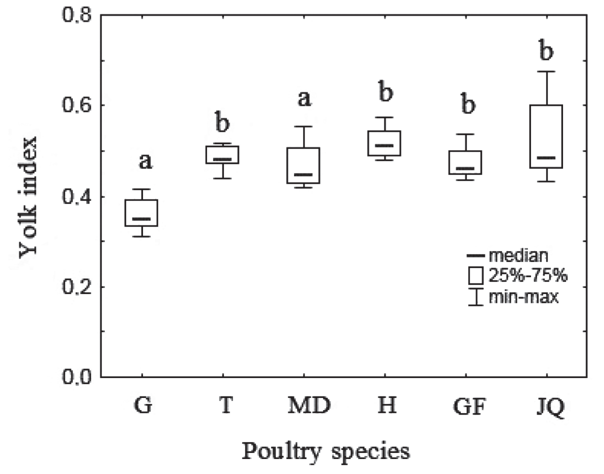

Figure 2. The yolk index of poultry eggs. Explanatory notes: a, b, values designated by the same letters do not differ statistically significantly ($P<0.05$, $n = 15$), WY – yolk weight, G – goose, T – turkey, MD – Muscovy duck, H – hen, GF – guinea fowl, JQ – Japanese quail

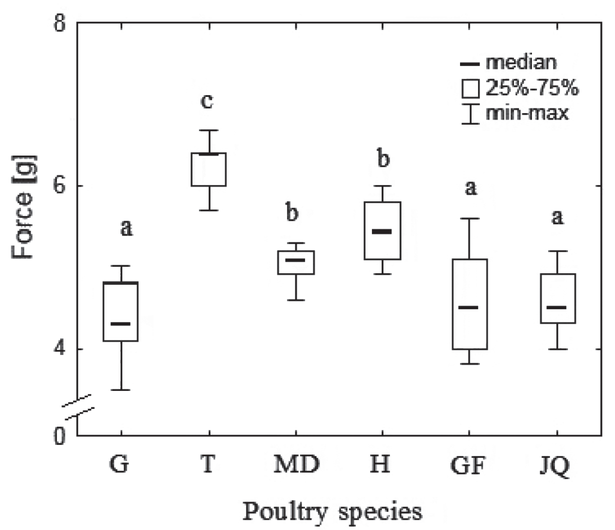

Figure 3. Force deformation of vitelline membrane of poultry eggs. Explanatory notes: a, b, values designated by the same letters do not differ statistically significantly ($P<0.05$, $n = 15$), WY – yolk weight, G – goose, T – turkey, MD – Muscovy duck, H – hen, GF – guinea fowl, JQ – Japanese quail

The vitelline membrane of turkey egg yolk (T) was characterized by the highest resistance to deformation at 6 mm, a median force was 6.4 g and the median of work was 0.18 mJ. The vitelline membrane strength (VMS) was significantly different from the strength of the membrane in other poultry species (Duncan test, P<0.05) (Figures 3, 4). Duck egg yolk (MD) and that of hens (H) were both characterized by a similar force and work. However, the lowest VMS values were obtained from the eggs of goose (G), guinea fowl (GF) and quail (JQ) (Figures 3, 4).

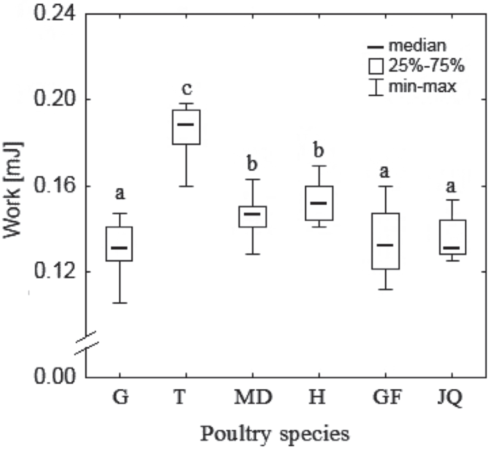

Figure 4. Work deformation of vitelline membrane of poultry eggs. Explanatory notes: a, b, c values designated by the same letters do not differ statistically significantly (P<0.05, n = 15), WY – yolk weight, G – goose, T – turkey, MD – Muscovy duck, H – hen, GF – guinea fowl, JQ – Japanese quail

In order to detect correlations between the strength of vitelline membrane and morphological parameters and the viscosity of the yolk in the eggs of different poultry species, a statistical analysis of the principal components (PCA) with classification was performed. Using the criterion of sufficient proportions and the Kaiser criterion (Stanisz, 2007), the number of components was reduced to two, which together accounted for 92.8% of the variation. The first component (PC1) was created by the force (F) and work (W) and yolk index (Ix), and it was called the strength component. PC1 explained 47.87% of the variance. The second component (PC2) was created by the weight (WY) and the apparent viscosity (VY) of egg yolk and accounted for 44.54% of the variance (Figure 5).

On the PCA map, the position of samples close to each other reflects their similarity, while the distance reflects the differences between yolks of the studied poultry species (Figure 5). Egg yolk of hens (H), guinea fowl (GF) and Muscovy ducks (MD) formed one group (1) characterized by a similar strength of vitelline membrane as well as weight and viscosity. Egg yolk of turkeys (T) and guinea fowls (GF) formed distinct groups on the PCA map, they were similar to the first group (H, GF and MD) in terms of PC1, but differed in terms of PC2 (weight and viscosity). In contrast, goose egg yolk (G) on the right side of the PCA map, had smaller VMS than the yolk of other poultry species, but similar PC2 (weight and viscosity) to the yolk of turkey eggs (T). Strong positive correlations were observed between the strength parameters of the vitelline membrane: strength and work and yolk index (Table 3). In contrast, a negative correlation was observed between the yolk index and viscosity. This paper does not state any correlation between the strength of the vitelline membrane and egg and yolk weight (Table 3).

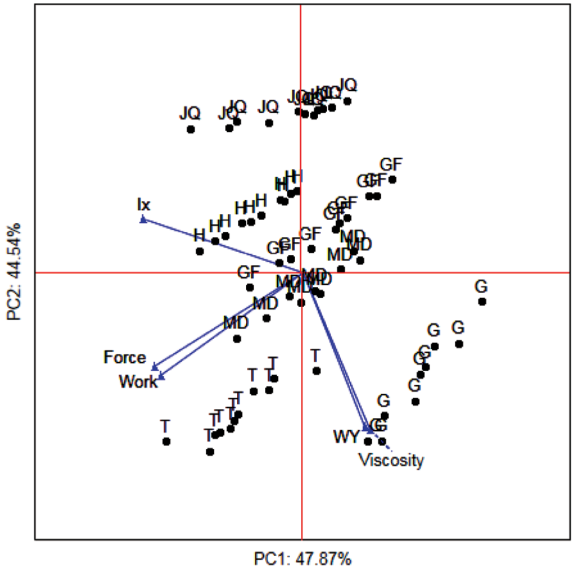

Figure 5. PCA map of similarities and differences between egg yolks of domestic birds. Ix – yolk index, WY – yolk weight, G – goose, T – turkey, MD – Muscovy duck, H – hen, GF – guinea fowl, JQ – Japanese quail

Table 3. Correlation between the strength of the vitelline membrane, viscosity and morphological characteristics of different species of poultry

	WE	WY	Ix	Force (g)	Work (mJ)
WY	0.968*	1.000			
Ix	–0.593*	–0.505*	1.000		
Force (g)	0.070	0.134	0.565*	1.000	
Work (mJ)	0.107	0.182	0.525*	0.973*	1.000
Viscosity (Pa·s)	0.855*	0.797*	–0.498*	0.214	0.298*

* Statistically significant correlations between parameters at $P<0.05$.
WE – egg weight; WY – yolk weight; lx – yolk index.

Discussion

Internal egg parameters such as yolk weight are very important from both nutritional and health points of view (Sparks, 2006; Kabir et al., 2012). In this regard, a high percentage by weight of the egg yolk in the entire weight of the egg should be considered preferable. According to literature data, the proportion of yolk was 33.94% in duck egg weight (Kaewmanee et al., 2009), and 28–29% in hen eggs (Kabir et al., 2012). According to the standard for hen eggs, yolk represents 31.9% of total egg weight, this value is higher than that obtained in this paper. However, long-term breeding work towards increasing the laying efficiency and egg weight has led to changes in the proportions of egg composition, resulting in a smaller share of yolk, particularly in commercial lines of laying hens as, for example, ISA Brown. Generally, the ratios of egg components are dependent upon the species and age of the poultry (Samli et al., 2005).

A hypothesis that eggs of different species of poultry have different strength of yolk vitelline membrane (VMS) has been confirmed. However, only a goose egg yolk had different VMS from the yolk of other poultry species. According to other authors, VMS is affected by the origin of birds, their age, time and storage conditions of eggs (Berardinelli et al., 2008; Jones et al., 2014), feeding, membrane structure (histological structure) resulting from these factors and its chemical composition. The structure and thickness of the vitelline membrane is different for duck and hen egg yolk. The widths of the fibrils shown in the outer layer and inner layer of duck egg vitelline membrane were 0.1 and 0.7−1.4 μm, respectively, and were greater than those (<0.1 and 0.5−0.8 μm) of hen egg vitelline membrane. The continuous membranes of both hen and duck egg vitelline membrane strength were still attached to the outer layers when separated. The content of protein, the major component of VM, was higher in duck egg VM (88.6%) than in hen egg VM (81.6%) (Chung et al., 2010). The present literature provides no information on the structure of the vitelline membrane in eggs of other poultry species.

However, studies show that the main reason for the differences in the strength of VMS can be yolk viscosity resulting from different chemical composition of the yolk. Apparent yolk viscosity as a physical indicator depends on the age of an egg, storage temperature, the pH value, specific weight, water content and egg stress. According to literature data, the highest dry matter content and the lowest water content are reported for ducks egg yolk (43.5–44.5%) (Kaewmanee et al., 2009) and that of turkey (46.2–46.6%) (Mróz et al., 2014). A much higher water content was found in goose egg yolk (48.6%) (Mazanowski, 2012), quail eggs (49.7%), guinea fowl eggs (49.8%) (Song et al., 2010) and hen eggs (55.7% – control conventional battery and 57.3% – free-range farming system) (Radu-Rusu et al., 2014). In addition, the intensity of lay has a decisive influence on the viscosity of the egg yolk. It probably would result in a significantly higher viscosity of egg yolks in geese and turkeys than the viscosity of egg yolk in ducks, guinea fowls or chickens and quails. Most likely, the lowest egg yolk viscosity in the case of hen and quail may be associated with a much higher laying performance of birds used for the mass production of table eggs. By comparison, poultry species which have not undergone intensive selection for egg laying, produce fewer eggs and are intended primarily for reproduction.

The yolk with a low index had high viscosity and such eggs' vitelline membrane was characterized by low strength. A significant correlation was also found between work and viscosity, which is dependent on the chemical composition of the yolk. Also Kirunda and McKee (2000) showed that the viscosity of yolks correlated quite well with their VMS. It can be assumed that the strength of the vitelline membrane and apparent viscosity of yolk are equally significant in ensuring yolk quality. Especially since recent literature reports indicate that the susceptibility of the viscosity to storage duration is much higher than that described in terms of the most often used parameters like Haugh units, pH of albumen and egg yolk index (Samli et al., 2005; Severa et al., 2010; Kumbár et al., 2015). The main constituents of egg yolk are triacylglycerols, phospholipids, proteins, and carbohydrates (Severa et al., 2010).

In summary, the strength of the vitelline membrane in poultry egg yolk is significantly affected by poultry species. The lowest strength was measured for the vitelline membrane of goose egg yolk. There were no apparent differences in the strength of vitelline membrane for turkeys, ducks, guinea fowl, hens and Japanese quails. A statistically significant positive correlation was observed between the strength parameters of the vitelline membrane (force and work) and yolk index, while there was no correlation with the weight of the eggs and egg yolk. The work was positively correlated with the viscosity of poultry egg yolk.

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